

**PATENT APPLICATION**  
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of

James E. GALEN

Appln. No.: 09/993,292

Confirmation No.: 5386

Filed: November 23, 2001

For: USE OF ClyA HEMOLYSIN FOR EXCRETION OF PROTEINS



Docket No: A8461

Group Art Unit: 1645

Examiner: Duffy, P.

**SUBMISSION OF EXECUTED DECLARATION UNDER 37 C.F.R. §1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Submitted herewith is a copy of an executed Declaration Under 37 C.F.R. §1.132 signed  
by James E. GALEN.

SUGHRUE MION, PLLC  
Telephone: (202) 293-7060  
Facsimile: (202) 293-7860

WASHINGTON OFFICE

**23373**

CUSTOMER NUMBER

Respectfully submitted,

Drew Hissong  
Registration No. 44,765

Date: July 11, 2005

**Best Available Copy**

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

James B. GALEN

Appl. No.: 09/993,292

Confirmation No.: 5386

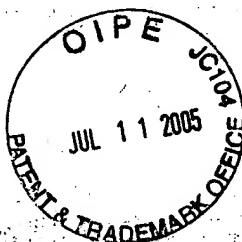
Filed: November 23, 2001

For: USE OF C1yA HEMOLYSIN FOR EXCRETION OF PROTEINS

Docket No: A8461

Group Art Unit: 1645

Examiner: Duffy, P.



SECOND DECLARATION OF JAMES E. GALEN UNDER 37 C.F.R. §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Sir,

I, James B. Galen, hereby declare and state:

THAT I am a citizen of the United States of America;

THAT I have received the degree of Ph.D. in 1991 from the University of Maryland

Baltimore;

THAT I have been employed by the Center for Vaccine Development since 1993, where I hold a position as Associate Professor, with responsibility for engineering expression systems for attenuated *Salmonella enterica* serovar Typhi human live vector vaccine strains.

Comments regarding inclusion of sequences in application

Paragraph 0028 of U.S. application number 09/993,292 refers to four polynucleotide sequences encoding bacterial proteins by name and GENBANK accession number. Namely:

The polynucleotide encoding *Shigella flexneri* truncated HlyE - Accession No. AF200955

The polynucleotide encoding *Escherichia coli* hlyE - Accession No. AJ001829

The polynucleotide encoding *Salmonella paratyphi clyA* - Accession No. AJ313033

The polynucleotide encoding *S. Typhi clyA* - Accession No. AJ313034

Each of the four polynucleotides was submitted to GENBANK and assigned an accession number prior to the date on which the present application was filed. None of the polynucleotide sequences is known to have been changed in the period of time between the filing of the present application (November 23, 2001), and the date of execution of the instant Declaration.

Furthermore, based on reasonable investigation, there is no evidence that any of the sequences have been changed during this period of time. Indeed, as shown on the attached GENBANK printouts for each of the four sequences (Appendix I-IV), there were no changes to the sequences under the relevant accession numbers since the filing date (November 23, 2001) of the pending application. With regard to AF200955, the last date of amendment was February 2, 2000 (Appendix I). With regard to AJ001829, the last date of amendment was April 2, 1998 (Appendix II).

With regard to AJ313033, the last date of amendment was April 15, 2005 (Appendix III). However, as shown on the enclosed summary of amendments made to the listing (Appendix V), the only changes that have been made are those to the classification of the organism, the references citing the accession number, and the addition of a SwissPro reference number (please see the boxed regions on Appendix V). No changes have been made to the sequence.

With regard to AJ313034, the last date of amendment was April 15, 2005 (Appendix IV). However, as shown on the enclosed summary of amendments made to the listing (Appendix VI), the only changes that have been made are those to the classification of the organism, the

SECOND DECLARATION OF JAMES E. GALEN UNDER 37 C.F.R. §1.132  
U.S. Appl. No. 09/993,292

A8461

references citing the accession number, and the addition of a SwissPro reference number (please see the boxed regions on Appendix VI). No changes have been made to the sequence.

As such, I aver that the reference material referred to herein contains no new matter and consists of the same material incorporated by reference into the above-identified U.S. patent application at the time the application was filed.

\*

\*

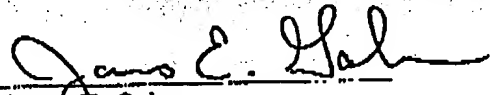
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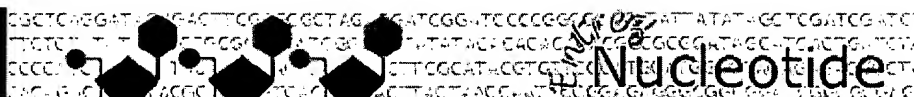
I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date:

7/11/05

  
James E. Galen

## APPENDIX I

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Range: from  to 
☐ Reverse complemented strand
 Features:
 ☐ SNP
 ☐ CDD
 ☒ MGC
 ☐ HPRD
 ☐ STS

**1: AF200955. Reports *Shigella flexneri*...[gi:6478772]**

[Links](#)

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 DEFINITION *Shigella flexneri* HlyE (hlyE) gene, complete cds.  
 ACCESSION AF200955  
 VERSION AF200955.1 GI:6478772  
 KEYWORDS .  
 SOURCE *Shigella flexneri*  
 ORGANISM *Shigella flexneri*  
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;  
 Enterobacteriaceae; *Shigella*.  
 REFERENCE 1 (bases 1 to 904)  
 AUTHORS Wallace,A.J., Stillman,T.J., Atkins,A., Jamieson,S.J.,  
 Bullough,P.A., Green,J. and Artymiuk,P.J.  
 TITLE *E. coli* hemolysin E (HlyE, ClyA, SheA): X-ray crystal structure of  
 the toxin and observation of membrane pores by electron microscopy  
 JOURNAL Cell 100 (2), 265-276 (2000)  
 PUBMED 10660049  
 REFERENCE 2 (bases 1 to 904)  
 AUTHORS Green,J.  
 TITLE HlyE of *Shigella flexneri*  
 JOURNAL Unpublished  
 REFERENCE 3 (bases 1 to 904)  
 AUTHORS Green,J.  
 TITLE Direct Submission  
 JOURNAL Submitted (02-NOV-1999) MBB, University of Sheffield, Western Bank,  
 Sheffield S80 4NB, UK  
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Feb 9 2005 14:31:10



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PubMed

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Protein

Genome

Structure

PMC

Taxonomy

OMIM

Books

Search

Nucleotide

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display

GenBank

Send

all to file

Range: from

begin

to

end

☐ Reverse complemented strand

Features:

☐ SNP

☐ CDD

☒ MGC

☐ HPRD

☐ STS

☐ 1: [AJ001829](#). Reports *Escherichia coli* ...[gi:3021363]

Links

LOCUS ECDNACLYA 1080 bp DNA linear BCT 02-APR-1998

DEFINITION *Escherichia coli* clyA gene.

ACCESSION AJ001829

VERSION AJ001829.1 GI:3021363

KEYWORDS clyA gene, Hemolysin.

SOURCE *Escherichia coli*

ORGANISM *Escherichia coli*  
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;  
Enterobacteriaceae; *Escherichia*.

REFERENCE 1

AUTHORS Ludwig,A., Bauer,S., Benz,R. and Goebel,W.

TITLE Molecular analysis of a latent pore-forming 34-kDa haemolysin from *Escherichia coli* K-12

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 1080)

AUTHORS Ludwig,A.

TITLE Direct Submission

JOURNAL Submitted (30-SEP-1997) Ludwig A., Lehrstuhl fuer Mikrobiologie, Universitaet Wuerzburg, Theodor-Boveri-Institut (Biozentrum), Am Hubland, D-97074 Wuerzburg, GERMANY

FEATURES Location/Qualifiers

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ORIGIN


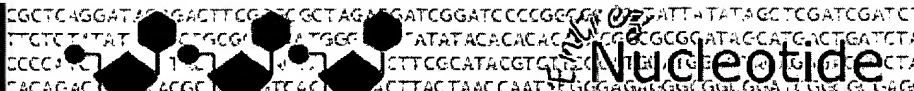
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☐ Reverse complemented strand
 Features:
 ☐ SNP
 ☐ CDD
 ☒ MGC
 ☐ HPRD
 ☐ STS

**1: AJ313033**. Reports *Salmonella paratyphi*...[gi:14140227]

[Links](#)

LOCUS SEN313033 1102 bp DNA linear BCT 15-APR-2005  
 DEFINITION *Salmonella paratyphi* clyA gene for cytolysin A.  
 ACCESSION AJ313033  
 VERSION AJ313033.1 GI:14140227  
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 SOURCE *Salmonella paratyphi* (*Salmonella enterica* subsp. *enterica* serovar Paratyphi A)  
 ORGANISM *Salmonella paratyphi*  
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; *Salmonella*.  
 REFERENCE 1  
 AUTHORS Oscarsson, J.  
 JOURNAL Thesis (1999) Department of Microbiology, Umea University, Umea, Sweden  
 REFERENCE 2  
 AUTHORS Oscarsson, J., Westermarck, M., Lofdahl, S. and Uhlin, B.  
 TITLE Expression of a pore-forming cytotoxin by *Salmonella* ser. Typhi and *Salmonella* ser. Paratyphi A  
 JOURNAL Unpublished  
 REFERENCE 3 (bases 1 to 1102)  
 AUTHORS Oscarsson, J.  
 TITLE Direct Submission  
 JOURNAL Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea University, Umea, 90187, SWEDEN  
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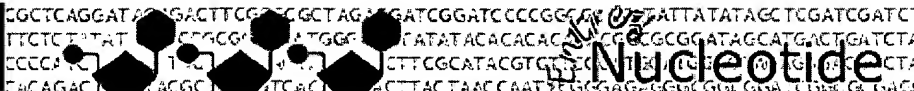
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Range: from  to 
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 Features:
 ☐ SNP
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 ☐ HPRD
 ☐ STS

**1: AJ313034. Reports Salmonella typhi ...[gi:14018374]**

[Links](#)

LOCUS SEN313034 1102 bp DNA linear BCT 15-APR-2005  
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 ACCESSION AJ313034  
 VERSION AJ313034.1 GI:14018374  
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 ORGANISM Salmonella enterica subsp. enterica serovar Typhi  
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;  
 Enterobacteriaceae; Salmonella.

REFERENCE 1  
 AUTHORS Oscarsson, J.  
 JOURNAL Thesis (1999) Department of Microbiology, Umea University, Umea, Sweden

REFERENCE 2  
 AUTHORS Oscarsson, J., Westermarck, M., Lofdahl, S. and Uhlin, B.  
 TITLE Expression of a pore-forming cytotoxin by Salmonella ser. Typhi and Salmonella ser. Paratyphi A  
 JOURNAL Unpublished

REFERENCE 3 (bases 1 to 1102)  
 AUTHORS Oscarsson, J.  
 TITLE Direct Submission  
 JOURNAL Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea University, Umea, 90187, SWEDEN

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ORIGIN

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//

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14140227

1

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LOCUS	SEN313033	1102 bp	DNA	linear	BCT 15-APR-2005
LOCUS	SEN313033	1102 bp	DNA	linear	BCT 15-MAY-2001

DEFINITION Salmonella paratyphi clyA gene for cytolysin A.

ACCESSION AJ313033

VERSION AJ313033.1 GI:14140227

KEYWORDS clyA gene; cytolysin A.

SOURCE Salmonella paratyphi (Salmonella enterica subsp. enterica serovar Paratyphi A)

SOURCE Salmonella paratyphi

ORGANISM Salmonella paratyphi

REFERENCE 1 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Salmonella.

REFERENCE 1 Bacteria; Proteobacteria; gamma subdivision; Enterobacteriaceae; Salmonella.

AUTHORS Oscarsson, J.

JOURNAL Thesis (1999) Department of Microbiology, Umea University, Umea, Sweden

REFERENCE	2
REFERENCE	2 (bases 1 to 1102)

AUTHORS Oscarsson, J., Westermarck, M., Lofdahl, S. and Uhlin, B.

TITLE Expression of a pore-forming cytotoxin by Salmonella ser. Typhi and Salmonella ser. Paratyphi A

JOURNAL Unpublished

REFERENCE 3 (bases 1 to 1102)

AUTHORS Oscarsson, J.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea University, Umea, 90187, SWEDEN

FEATURES Location/Qualifiers

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14018374 1 Apr 16 2005 12:40 AM  
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LOCUS	SEN313034	1102 bp	DNA	linear	BCT 15-APR-2005
LOCUS	SEN313034	1102 bp	DNA	linear	BCT 09-MAY-2001

DEFINITION Salmonella typhi clyA gene for cytolysin A.

ACCESSION AJ313034

VERSION AJ313034.1 GI:14018374

KEYWORDS clyA gene; cytolysin A.

SOURCE Salmonella enterica subsp. enterica serovar Typhi

ORGANISM Salmonella enterica subsp. enterica serovar Typhi

Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Salmonella.

REFERENCE 1

Bacteria; Proteobacteria; gamma subdivision; Enterobacteriaceae; Salmonella.

REFERENCE 1 (bases 1 to 1102)

AUTHORS Oscarsson, J.

JOURNAL Thesis (1999) Department of Microbiology, Umea University, Umea, Sweden

REFERENCE 2

REFERENCE 2 (bases 1 to 1102)

AUTHORS Oscarsson, J., Westermark, M., Lofdahl, S. and Uhlin, B.

TITLE Expression of a pore-forming cytotoxin by Salmonella ser. Typhi and

Salmonella ser. Paratyphi A

JOURNAL Unpublished

REFERENCE 3 (bases 1 to 1102)

AUTHORS Oscarsson, J.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2001) Oscarsson J., Molecular Biology, Umea

University, Umea, 90187, SWEDEN

FEATURES Location/Qualifiers

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CDS 75...986  
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ORIGIN

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